HEI Perspectives

April 2002

Insights from HEI's research programs

Understanding the Health Effects of Components of the Particulate Matter Mix: Progress and Next Steps

Particulate matter (PM) is a complex mixture of particles suspended in the air that vary in size and composition. Epidemiologic studies over the last decade have reported associations between short-term increases in exposure to PM and increases in morbidity and mortality, particularly among those people with respiratory or cardiovascular disease. On the basis of these findings, many governmental agencies have reevaluated regulatory standards or guidelines for levels of PM in the air.

Recent studies funded by HEI and other agencies have corroborated and extended the associations found in the earlier studies. The recent epidemiologic studies and studies of controlled exposure to PM in humans and other species have begun to provide information about critical issues in PM research: [A] the size and chemical composition of particles that may cause harmful human health effects, [B] the potential biologic mechanisms of PM effects that underlie the epidemiologic associations previously reported, and [C] the groups of people that may be particularly sensitive to the effects of PM. Progress has been made in addressing these issues. Nevertheless, to inform future regulatory discussions on control strategies, a systematic research effort is required to develop a better understanding of the health effects of different components of the PM mixture and the mechanisms of PM effects.

Introduction

Over the past decade, many epidemiologic studies using advanced statistical techniques have shown an association between exposure to small, *short-term* increases in PM levels and increases in daily mortality and symptoms of certain illnesses (1–3). For example, they have shown an increase in death due to respiratory and cardiovascular diseases and a worsening of symptoms in people with asthma. These "time-series" studies were conducted in a variety of locations; most studies measured PM levels below the regulatory standards set in the United States and Europe to protect the public's health and particularly those populations considered to be most at risk from the effects of PM (see Sidebar 1). "Cohort" studies (studies of specified populations) over prolonged time periods in different communities have reported associations between *long-term* PM exposure and increased death rates due to cardiovascular disease as well as an increased incidence of respiratory disease (4,5). Generally, in both short-term and long-term studies, the magnitude of the effect of PM exposure was small—much smaller than the effects of tobacco smoke on disease that have been reported in similar epidemiologic studies. Widespread exposure to particles, nevertheless, may significantly affect public health.

It has been known for decades that short-term exposure to high levels of PM air pollution is associated with increases in the number of deaths in the region (eg, in air pollution episodes such as the thick London fogs of the 1950s). However, the interpretation of data from the recent time-series studies, in which people were exposed to much lower levels of air pollution, has been debated. The first issue of *HEI Perspectives,* "Airborne Particles and Health: HEI Epidemiologic Evidence" (6), presented evidence from HEI-funded epidemiologic studies that addressed questions about the interpretations of the recent time-series studies.

The issues raised include: inadequate control in analyses for other risk factors that could affect mortality and may be correlated in time with PM exposure (such as weather, influenza epidemics, or other air pollutants), the use of area-wide measurements of exposure rather than individual measurements for each member of the study population, and the lack of supporting evidence from toxicologic studies. The first *HEI Perspectives* concluded that "epidemiologic evidence of PM's effects on mortality and morbidity persists even when alternative explanations have been largely addressed," but that many questions remain concerning the association between exposure to PM and adverse health effects.

The recent findings from time-series and other new studies are informing efforts by public agencies in the United States, Europe, and Asia to revise and/or retain their ambient air quality standards for inhalable particles. On the basis of these findings, those agencies are also taking initial regulatory actions to control particle emissions. Even as these reviews and regulatory actions are taking place, scientists and policy experts are focusing on a further set of key questions about the toxicity of different components and characteristics of the particle mixture, which vary depending on the source of the particles. Air quality regulations in the United States and other countries are generally based on measurements of particle mass in broad size ranges (Sidebar 1). In the longer term, standards that are more sharply focused (eg, to cover a narrower size range or to target specific chemical components) may be equally or more effective than broader standards in controlling emissions of toxic particles and may affect fewer types of sources of emissions. They also may enable certain industries to control specific components of emissions to meet a regulatory standard. Research by HEI and others is building knowledge to inform these future regulatory decisions.

This issue of *HEI Perspectives* considers results from recent epidemiologic and toxicologic studies funded by HEI and others to assess progress in answering the critical question: *What attributes of particles are associated with toxicity?* and the intertwined questions related to it: *How do particles cause adverse effects and which population subgroups are particularly susceptible?* It concludes by pointing out the need for a major systematic research effort aimed at understanding which PM sources pose the greatest risk to human health.

What Are the Sources of Airborne Particles?

The sources of PM are numerous; naturally occurring processes and human activities all contribute to total ambient PM. Naturally occurring PM includes dust from the earth's surface (crustal material), sea salt in coastal areas, and biologic material in the form of pollen, spores, or plant and animal debris. In some rural areas, periodic forest fires produce large amounts of PM. In urban environments, particles arise mainly as a result of combustion from mobile sources such as cars, buses, ships, trucks, and construction equipment, and from stationary

SIDEBAR 1. REGULATING LEVELS OF PM

Since 1971 the US EPA has set limits, known as the National Ambient Air Quality Standards (NAAQS), on daily and annual average concentrations of particles in the air. Since 1997, two sizes of particles have been regulated: $PM_{2.5}$ and PM_{10} (particles smaller than 2.5 μm and 10 μm in aerodynamic diameter, respectively; see text for further details). $PM_{2.5}$ is a subset of PM_{10} but is regulated separately to ensure that the smaller particles, which have less mass but may be more toxic, are adequately controlled.

The Table shows the current US standards set in 1997, standards set or proposed separately by the state of California, and the similar "Limit Values" set by the European Commission for its member states. These agencies have set *annual* and *daily* standards. For the EPA, for example, the annual standard is met when the 3-year average of the mean PM concentrations measured by monitors in a community is less than or equal to the number indicated; the daily standard is met when the 3-year average of the 98th or 99th percentile of 24-hour PM concentrations at each monitor in a community is less than or equal to the number indicated. Standards for PM, measured in many different ways, have also been set in Japan, China, India, and other countries.

PM Standards

	Time Period	PM_{10} ($\mu g/m^3$)	$PM_{2.5}$ ($\mu g/m^3$)
United States EPA[a]	Daily	150	65
United States EPA[a]	Annual	50	15
California	Daily	50	Under discussion in 2002
California	Annual	20	12 (Proposed in 2002)
European Union[b]	Daily	50	Not set
European Union[b]	Annual	20	Not set

[a] Under revision.

[b] To be met in 2010 (to be reviewed in 2003).

sources such as heating furnaces, power plants, and factories. Near highways, motor vehicle emissions may dominate the pollution mixture, but in other locations emissions from a power plant or steel mill may be the main source of particulate pollutants. A significant fraction of PM, referred to as *secondary particles,* is produced by chemical reactions in the atmosphere; nitrogen oxides, sulfur dioxide, and organic compounds react with ozone and other reactive molecules (including free radicals) to form nitrates, sulfates, and other particles.

People are also exposed to PM indoors, mostly from cigarette smoke, home heating sources (such as wood-burning stoves), and cooking, but also from outdoor PM sources that easily penetrate the indoor environment. Indoor exposure may be substantial because this is where most people spend the majority of their time. However, when outdoors, people tend to be more active, which increases respiration. Active people may inhale a larger amount of pollutants because they inhale a larger amount of air in any given time period than people who remain indoors. Studies by HEI and others are under way to better understand the contribution of indoor and outdoor sources of PM to total exposure.

What Are the Physical and Chemical Characteristics of Particles?

PM is a complex mixture of solid and liquid particles. This mixture can vary greatly in size, composition, and concentration, depending on the sources generating the particles and such factors as geographic location, season, day, and even time of day.

Size

The size of ambient air particles ranges over a wide scale, from approximately 0.005 to 100 μm in aerodynamic diameter (that is, from the size of just a few atoms to about the thickness of a human hair). Researchers have defined size categories of these particles differently. Figure 1 shows that the distribution of particles measured in urban air falls into three main *modes* based on their aerodynamic diameter: *nuclei* mode (smaller than about 0.1 μm), *accumulation* mode (between approximately 0.1 and 1 μm), and *coarse* mode (larger than 1 μm). Other definitions of these particles used in health effects studies and for regulation are: *ultrafine* particles, smaller than about 0.1 μm in aerodynamic diameter (corresponding in size to

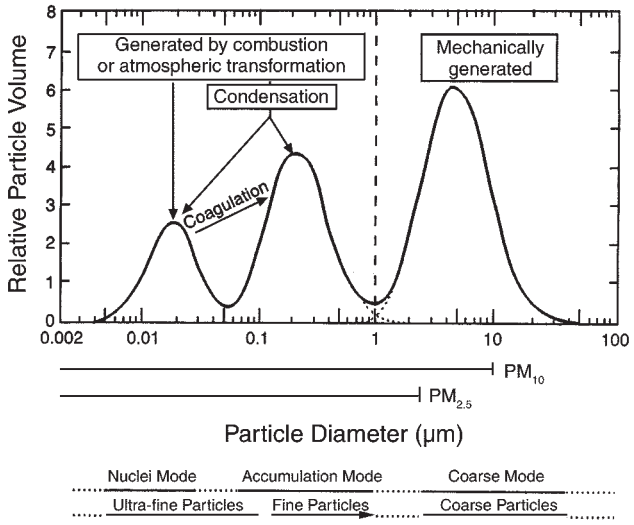

Figure 1. Typical distribution of three sizes or modes of particles in urban air and how different definitions of particle size relate to these modes (69).

nuclei-mode particles); and *fine* particles, smaller than 1 µm in aerodynamic diameter (containing all of the nuclei-mode and accumulation-mode particles). Finally, the categories currently used by the United States Environmental Protection Agency (EPA) and other agencies in the regulation of ambient PM are $PM_{2.5}$ and PM_{10}, which refer to particles with aerodynamic diameters smaller than 2.5 µm and 10 µm, respectively.

Ultrafine particles do not last long in the atmosphere. They tend to form fine particles either by coagulating (two or more small particles combining) or condensing (gas molecules condensing onto a solid particle). They are always present in the ambient air at some level, however, because they are constantly generated from combustion sources. Fine and ultrafine particles are formed mostly by emissions from combustion processes. By contrast, coarse particles are generated mainly by mechanical processes that break down material from a variety of noncombustion sources into dust.

As Figure 1 also illustrates, the largest particles (coarse particles in particular) form the highest proportion of the *mass* of ambient particles; the smallest, ultrafine particles, comprise only 1% to 8% of this mass. Ultrafine particles, however, are present in very high *numbers*, and, in a fixed volume, have greater *total surface area* than larger particles.

Size determines how likely different particles are to deposit in different parts of the respiratory tract. At the extremes, particles larger than about 10 µm in aerodynamic diameter are deposited almost exclusively in the nose and throat, whereas fine and ultrafine particles are able to reach the alveoli (air spaces) deep in the lungs. Generally, the smaller the particle, the greater the likelihood that it will penetrate deeper into the airways.

Fine and ultrafine particles also may carry toxic components into the deep lung. Because smaller particles are present in greater numbers, they have a greater total surface area than larger particles of the same mass; the toxic materials carried by small particles, especially ultrafine particles, may be more likely to interact with cells in the lung than those carried by larger particles. Thus, some scientists have proposed that ultrafine particles are especially toxic.

Composition

The composition of PM varies greatly and depends upon many factors, including source, climate, and the topography of the locale. In the United States, for example, nitrates tend to predominate in the west, whereas sulfates predominate in the east; in addition, sulfate levels are higher in summer than in fall or winter. Even in a single location the composition of PM can vary from year to year, season to season, day to day, and within a day.

Scientists have hypothesized that some components of ambient PM are more likely to be responsible for toxic or adverse health effects than other components. Table 1 lists some of the leading candidates that have been associated with biologic responses observed in a variety of studies and experimental systems. Studies are being conducted to determine which components or combination of components are key in inducing adverse health effects.

The major components of PM are metals, organic compounds, material of biologic origin, ions (that is, positively or negatively charged atoms), reactive gases, and the particle core (which is frequently composed of pure, or elemental, carbon). Table 1 also illustrates the composition and biologic effects attributed to secondary particles, a major subcomponent of the ion fraction. These particles are mostly composed of ammonium sulfate, ammonium nitrate, and secondary organic compounds that are produced in the atmosphere via reactions of gases with reactive organic compounds.

In general, the composition of larger particles differs from that of smaller particles. The coarse particle

Table 1. Chemical Components of PM and Their Biologic Effects

Component	Major Subcomponents	Described Biologic Effects
Metals	Iron, vanadium, nickel, copper, platinum, and others	Can trigger inflammation, cause DNA damage, and alter cell permeability by inducing production of reactive oxygen species (particularly hydroxyl free radicals) in tissues
Organic compounds	Many are adsorbed onto particles; some volatile or semivolatile organic species form particles themselves	Some may cause mutations, some may cause cancer; others can act as irritants and can induce allergic reactions
Biologic origin	Viruses, bacteria and their endotoxins (lipopolysaccharides), animal and plant debris (such as pollen fragments), and fungal spores	Plant pollens can trigger allergic responses in the airways of sensitive individuals; viruses and bacteria can provoke immune defense responses in the airways
Ions	Sulfate[a] (usually as ammonium sulfate), nitrate[b] (usually ammonium or sodium nitrate), and acidity (H^+)	Sulfuric acid at relatively high concentrations can impair mucociliary clearance and increase airway resistance in people with asthma; acidity may change the solubility (and availability) of metals and other compounds adsorbed onto particles
Reactive gases	Ozone, peroxides, aldehydes	May adsorb onto particles and be transported into lower airways, causing injury
Particle core	Carbonaceous material	Carbon induces lung irritation, epithelial cell proliferation, and fibrosis after long-term exposure

[a] Formed from the neutralization of sulfuric acid vapor, which is generated from the oxidation of sulfur dioxide emitted from combustion of fuel containing sulfur, such as that used in motor vehicles and oil- and coal-burning powerplants.

[b] Formed from nitric acid vapor, which is generated in the atmosphere from the reactions of nitrogen oxides.

fraction consists mainly of insoluble crust-derived minerals, biologic material (such as pollen and bacteria), and sea salts. By contrast, the ultrafine and fine fractions are composed mainly of particles with a carbon core that contains a variety of metals, secondary particles, and hydrocarbons.

How Does Exposure to Particles Affect the Cells and Tissues of an Individual?

The body responds to particulate pollution with the same multilayered defense system that it uses to defend itself against other foreign material such as bacteria and viruses: that is, by attempting to prevent access, and then by trying to rid the body of the foreign matter. Each succeeding layer brings stronger weapons into the battle. The first important level is a barrier of cells and fluids that the foreign material must get through before it enters the tissues of the body. Fluid secretions, such as mucus lining the airways, and ciliated cells are important elements of this system. Thus, many particles, especially larger ones, are trapped and removed by the nose. Coughing, activated by particles interacting with receptors on nerve cells in the airways, also helps to remove particles. In addition to these mechanisms, epithelial cells are tightly joined at the surface of a tissue, preventing material from entering between the cells.

If foreign material gets past these defenses and enters the tissue, a second line of defense comes into play: "scavenger" cells ingest the foreign material and attempt to destroy it. The most important scavenger cells are macrophages (white blood cells that reside in the tissues and in the airspaces of the lungs) and neutrophils (white blood cells found in the bloodstream that supplies the lung and other tissues). If the burden of foreign material overwhelms this line of defense, as can occur in bacterial or viral infections or in response to inhaled agents that cause allergic reactions, lymphocytes (another type of white blood cell) and the proteins they synthesize become involved in the response.

Particle deposition on the surface of epithelial cells in the airways and particle ingestion by macrophages and neutrophils is generally referred to as the *activation* of all these cells. Many of the effects that occur rapidly after particles impinge on airway cells are not fully understood. The activated cells are known to synthesize compounds referred to as reactive oxygen species (such as hydrogen peroxide) that try to eliminate the invading

foreign material. Within hours, proteins (called *cytokines*) and smaller molecules (called *chemokines*) are synthesized and secreted into the affected area. These molecules are mediators that interact with specific receptors on the surfaces of many cell types and result in activating cells in the surrounding environment as well as in the blood and other tissues. As a result, cells leave the bloodstream and enter the fluid-filled spaces of the airways (interstitial fluid), where they can attack the foreign material. Consequently, particle-induced cell activation events in the airways frequently result in an *inflammatory* response. This response includes both the activation of airway cells (including the production of the "proinflammatory" and reactive oxygen molecules described above) and the activation and migration of cells (particularly neutrophils and a related cell type, eosinophils) from the blood into the airways.

The inflammatory response may damage the epithelial cell layer at the surface of the tissue and other cells in the airway (such as macrophages), which results in the loss of integrity of the tissue's defenses. One potential consequence may be increased exposure to and reduced capacity to defend against microorganisms.

At least one of the cytokines produced by the inflammatory response in the airways stimulates the liver to secrete a set of molecules known as acute-phase reactants. These molecules, which include C-reactive protein and fibrinogen, appear in the circulation within 6 to 24 hours. Fibrinogen binds to platelets and contributes to their aggregation. This can result in multiple effects throughout the cardiovascular system, including an enhanced ability of the blood to clot (increased coagulability).

Although cytokines may have a primary role in inducing these nonpulmonary effects, some recent research also suggests that particles (and ultrafine particles in particular) or particle components may physically move out of the airways and rapidly into the bloodstream to trigger effects at distant sites.

Deposition of particles in the airways can stimulate nerve cells in the underlying tissue as well. Activation of these cells has been suggested to lead to changes in the nervous system's control of the pattern of breathing, the heart rate, and heart rate variability (a measure of the fluctuations in heart rate that occur in all individuals), and to affect other cardiac electrophysiologic parameters.

Thus, particle deposition in the airways can set off a cascade of events in many different cells, potentially resulting in changes in tissues and organs at sites progressively further away from the initial stimulus. These defense mechanisms are normal responses in healthy individuals, but they may lead to deleterious changes in the host. Such changes may be rapid and temporary and may resolve quickly; but depending on the level and pattern of exposure and the agent to which the host is exposed, the changes may last longer. It is not clear whether or how such changes are relevant to the development of PM-induced adverse health effects at low levels of exposure. These changes are thought to have a greater impact on individuals whose airway, cardiac, or vascular tissues have been previously damaged.

What Have We Learned from Recent HEI-Funded and Other Studies About the Effects of Particles?

Many agencies have funded a vast amount of epidemiologic and experimental research on the health effects of PM. The US EPA and the California Air Resources Board are in the latter stages of extensive multistep reviews of recent PM studies. Their aim is to determine whether recent findings necessitate modifying PM regulatory standards in the United States as a whole or in California, respectively. These reviews will be published in final form in the coming months. The European Union, through the World Health Organization's European Centre for Environment and Health, also has recently initiated a similar review. Rather than repeat the extensive literature cited in the reviews from these regulatory agencies, the following paragraphs summarize some of the key information about PM health effects and highlight studies that have been particularly informative.

HEI's recent PM research program has funded and continues to fund studies focused on three key topics:

1. the relative toxicity of different components of the PM mixture;
2. induction pathways for adverse effects; and
3. identifying groups in the population who may be particularly susceptible to PM effects.

Table 2 shows how these three critical areas have been addressed in both completed and ongoing HEI PM studies. The studies evaluated a range of endpoints, some representing true health effects (such as asthma symptoms or mortality), whereas others measured early phenomena (such as production of inflammatory mediators and changes in gene expression or heart rate). Many of the funded studies addressed questions in more than one of these areas. Sidebar 2 describes the range of approaches that have been taken in humans and other species, providing insight into identifying toxic components of the complex PM mixture and the effects of different types of PM.

What Attributes of Particles Are Associated with Toxicity?

Studies have investigated the physical aspects (particularly size) and the chemical composition of particles that induce effects in humans and other species. Several recent epidemiologic studies (8–14) in different locations have reported associations between various health effects and different sizes and/or chemical components of the particles to which the study populations had been exposed. Findings differed from study to study, generally depending on where and when the study was conducted. For example, some epidemiologic studies in Mexico City and the western United States have found health effects associated with the coarse particle fraction (11,12), but studies conducted in other parts of the United States and in Canada have reported that effects of fine particles predominate (8–10). In a recent study in Germany, levels of both fine and ultrafine particles were associated with increased mortality (16). Several reasons may be suggested for such discrepancies among studies: [1] the nature of PM varies in different regions with different sources, climate, and topography; [2] studies use a variety of statistical methods to assess results; [3] studies may use different measurements of PM (eg, $PM_{2.5}$ vs ultrafine particles); and [4] studies may have different health endpoints.

The HEI-funded* National Morbidity, Mortality, and Air Pollution Study (NMMAPS), directed by *Jonathan Samet*, investigated the association of PM_{10} with mortality using a unified method in the 90 largest US cities (15). This study was designed to address many of the criticisms raised about the time-series studies that had shown an association between short-term increases in PM levels and increases in the number of deaths. (Two main criticisms were that the analytic methods had varied among studies and that the lack of criteria for selecting cities to study may have led to bias in the results.) NMMAPS found that a 10-μg/m^3 increase in PM_{10} resulted in an average increase of about 0.5% in mortality from all causes. Adding other pollutants to the model did not appear to affect the result found with PM_{10}. Regional differences in the PM_{10} effect were seen, with the largest effects evident in the northeastern United States. One explanation for the regional differences may be variation in the nature of PM; because it is a complex mixture, the same mass of PM_{10} in different places may include very different amounts of fine or ultrafine particles and particles with different composition (eg, higher presence of some metals).

Size

In their HEI study, *Erich Wichmann* and colleagues characterized the sizes of particles in the ambient air of Erfurt, Germany, and determined whether they were related to changes in daily mortality (16). They reported that over a three-year period the concentrations of both ultrafine ($PM_{< 0.1}$) and fine particles ($PM_{0.1–2.5}$) were associated with increased daily mortality. These findings provided the first evidence that ultrafine particles were associated with human mortality, but did not indicate whether ultrafine particles were more toxic than larger particles.

In another HEI study, *Morton Lippmann* and colleagues compared day-to-day fluctuations in hospital admissions of older people and deaths in the Detroit-Windsor area with day-to-day fluctuations in levels of different ambient PM size fractions (17). They found that four of the five size fractions they evaluated were associated with increased morbidity and mortality. These were total suspended particles (TSPs; ie, all particle types and sizes up to about 40 μm in aerodynamic diameter found in ambient air); PM_{10}; $PM_{2.5–10}$ (ie, particles between 2.5 μm and 10 μm in aerodynamic diameter); and $PM_{2.5}$. The magnitude of the association was similar for all four fractions. The largest particle size fraction (between 10 μm and about 40 μm) was not associated with increased morbidity and mortality. The investigators also reported that the particles fractionated by size were more significantly associated with health outcomes than were the two chemical components of ambient PM, acidity and sulfate, evaluated in the study.

* HEI-funded studies are indicated by italic type on the Principal Investigator's name.

Table 2. Questions Addressed by HEI-Funded Studies[a]

Study Description	Groups Studied	Possible Mechanisms	Particle Aspects
Epidemiology			
Samet et al (15) found that PM_{10} was associated with increased mortality in the 90 largest US cities. In a smaller subset of cities, PM_{10} was also associated with increased hospital admissions of the elderly.	General population, elderly	NA	PM_{10}
Goldberg et al (56) found, in Montreal, that coefficient of haze, sulfate, and $PM_{2.5}$ were associated with mortality from respiratory disease and diabetes. The study also showed that people with neither cardiovascular nor pulmonary disease were at increased risk of mortality from PM.	General population to identify susceptible groups	NA	NA
Wichmann et al (16) found, in Germany, that both ultrafine (< 0.1 μm) and fine particles (0.1–2.5 μm) were associated with increased daily mortality, but the findings did not show a consistent pattern regarding relative toxicity.	General population	NA	Size (fine, ultrafine)
Lippmann et al (17) found, in Detroit-Windsor, associations between different size fractions of PM and deaths and hospital admissions of the elderly. All size fractions except the largest (particles larger than PM_{10}) showed an association; the associations with particle size were stronger than those for acidity or sulfate content.	General population, Elderly	NA	Size (fine to coarse), sulfate, acidity
Checkoway et al (53) found, in Seattle, no association between PM and sudden cardiac arrest in people with no known history of heart disease. This suggests that these people are not at increased risk.	General population	NA	$PM_{2.5}$, PM_{10}
Ongoing: Peters et al are studying, in Germany, the possible association between PM and incidence of nonfatal myocardial infarction.	People with an incidence of myocardial infarction	Cardiac	Size (ultrafine, fine)
Ongoing: Dockery et al are studying, in Boston, the association between PM and triggering of implanted cardiac defibrillators to stop serious cardiac arrhythmia.	Cardiac disease	Cardiac	$PM_{2.5}$, PM_{10}, carbon
Human Controlled Exposure			
In review: Holgate et al (70) collected bronchial biopsy tissue from healthy and asthmatic subjects exposed to diesel exhaust or CAPs in Research Triangle Park. Inflammatory markers increased in healthy but not asthmatic subjects after diesel exposure and not in healthy subjects after CAPs exposure; CAPs were not studied in asthmatic subjects.	Healthy, asthma	Inflammation	Diesel, CAPs
Ongoing: Frampton et al are exposing healthy and asthmatic subjects to ultrafine PM and evaluating their lung function, inflammatory markers in sputum, and vascular and electrophysiologic cardiac markers.	Healthy, asthma	Cardiac, vascular or inflammatory	Ultrafine carbon
Ongoing: Gong et al are exposing healthy and asthmatic subjects to CAPs in Los Angeles and evaluating lung function, inflammatory markers in sputum, and vascular and electrophysiologic cardiac markers.	Healthy, asthma	Cardiac, vascular or inflammatory	CAPs
Animal and in Vitro Exposures			
Oberdorster et al (18) found that inhaling ultrafine carbon and platinum particles for a short time induced an inflammatory response in the airways of mice and rats with pulmonary disease but not in healthy young or healthy old rodents.	Rodents: Pulmonary disease, healthy young, healthy old	Inflammation	Ultrafine carbon, platinum

(*Table continues next page*)

[a] NA = Not applicable.

Table 2 (*continued*). Questions Addressed by HEI-Funded PM Studies

Study Description	Groups Studied	Possible Mechanisms	Particle Aspects
Animal and in Vitro Exposures (*continued*)			
Godleski et al (28) found, in Boston, that inhalation of CAPs changed the ECG in dogs with an induced temporary heart condition; in healthy dogs, they found changes in heart rate variability of uncertain significance and little change in inflammatory markers.	Dogs: Healthy, cardiac condition	Nervous system control of heart	CAPs
Vincent et al (42) found, in Ottawa, increased blood pressure and vascular factors called endothelins in healthy rats exposed by inhalation to resuspended urban PM. Effects were less with washed PM and diesel particles; no effects were evident with carbon, which indicates a role for soluble components such as metals.	Rats: Healthy	Vascular	Urban PM, washed PM, diesel exhaust, carbon
Kobzik et al (54) evaluated, in Boston, inhalation of ozone and CAPs on pulmonary function and airway inflammation in young healthy and "asthmatic" mice. Effects on pulmonary function and airway inflammation were small; some, but not all, the statistical models used suggested synergy between CAPs and ozone; CAPs collected on filters stimulated airway macrophage cytokine production in vitro.	Mice: Healthy young, "asthmatic" young	NA	CAPs
Leikauf et al (20) used genetic and molecular approaches to characterize genes that may be involved in genetic susceptibility of mice to prolonged nickel inhalation; two candidate genes identified were those coding for surfactant-associated protein B and transforming growth factor α.	Mice: Susceptibility to death from continuously inhaled nickel	Lung injury or inflammation or both	Nickel
Gordon et al (68) studied, in New York City, inhaled CAPs in healthy and hypertensive rats and in guinea pigs with a cardiac condition (small numbers of animals and low CAPs concentrations). No groups showed inflammatory changes.	Rats: Hypertensive; Guinea pigs: Cardiac condition	Cardiac	CAPs
In press: Aust et al (71) studied the impact of metals on cultured epithelial cells and found that metals can detach from the particles and, once inside the cell, stimulate production of inflammatory mediators.	NA	Inflammation	Metals
In press: Laskin et al (76) evaluated whether exposing rats to an inhaled combination of aerosolized ammonium sulfate and hydrogen peroxide induced more lung injury than did the individual components. Compared with the individual components, the combination increased only some of the parameters measured.	Rats	Lung injury or inflammation or both	Aerosolized ammonium sulfate and hydrogen peroxide
In press: Nadziejko et al (77) found, in New York City, no effect of inhaling CAPs on blood coagulation parameters in small numbers of healthy rats with low CAPs concentrations.	Rats: Healthy	Blood coagulation	CAPs
Ongoing: Hahn at al are examining the effects of inhaling fine and ultrafine vanadium pentoxide and carbon particles on lung inflammation and pathology in old rats and rats with airway inflammation.	Rats: Airway inflammation, healthy old	Lung injury or inflammation or both	Fine and ultrafine carbon and vanadium
Ongoing: Pinkerton et al are examining the effects of inhaling ultrafine iron particles in combination with carbon particles on the lungs of young and adult rats and looking at the pattern of injury in the lower lung.	Rats: Young, adult	Lung injury or inflammation or both	Ultrafine; iron, cerium, carbon
Ongoing: Witten et al are investigating in rats the effects of inhaling diesel exhaust on lung injury (such as decreased pulmonary function and increased airway inflammation) and the role of sensory nerve cells in mediating the inflammatory response (neurogenic inflammation).	Rats: Healthy	Neurogenic inflammation	Diesel exhaust

SIDEBAR 2. INVESTIGATING MECHANISMS OF PM HEALTH EFFECTS

If we knew which properties of PM were responsible for toxicity, emissions and air quality standards could then focus on controlling the particles doing the most damage. However, the PM mixture is extremely complex, so trying to identify which components of PM cause a particular adverse effect is extremely challenging. In addition, ambient air contains gaseous pollutants such as ozone that can exert adverse effects similar to those ascribed to components of PM. The following describes the strengths and limitations of different approaches that have been taken to address the special challenges in studying the effects of exposure to ambient PM.

1. *Evaluating responses to different types of particles*. Studies have evaluated exposure to (a) ambient particles (in particular, concentrated "fresh" ambient particles or resuspended stored particles); (b) particles from specific sources (such as oil and coal fly ash, diesel exhaust, gasoline exhaust); and (c) particles generated in the laboratory (for example, metals or metal oxide particles).

Each of these methods can address different aspects of PM effects. Fresh ambient particles of different sizes have the positive aspect of containing the "right stuff" (ie, what has been associated with adverse health effects in epidemiologic studies), but because of the complexity of ambient PM, it is very difficult to identify which components are influencing the effects. For this reason, particles from specific sources, which are themselves complex mixtures but less so than the ambient mixture, may also be useful. This approach makes sense because sources are the focus of emissions controls. One drawback, however, is that often the particles are collected, stored, and then later resuspended for exposure to animals or cells in culture; thus, their physical properties are likely to have altered and their chemical properties may have changed as well. A second problem is that particles gathered from a source may not be generally representative of that source. For example, particles in the air derived from a large number of motor vehicles (with varying emissions characteristics) may not be well represented in studies using samples from one or a few test engines. The third approach, using "model" particles generated in the laboratory to test specific hypotheses about size and composition is useful in sorting out hypotheses, but cannot be used to test all variations in ambient PM. Moreover, these particles may not be representative of ambient particles.

2. *Choosing appropriate endpoints of PM effects*. Researchers have evaluated multiple endpoints in completed and ongoing epidemiologic and experimental studies. These endpoints include health effects, such as mortality and the incidence of cardiovascular illness, and early cellular and tissue events after PM exposure, such as changes in cytokine levels or in heart rate variability (see text for further details).

The choice of appropriate endpoints of PM effects is especially challenging because the mechanisms by which particles may cause health effects are not known with certainty, and it is also possible that different types of particles act by different mechanisms. In addition, as described above, not all the biologic responses measured may be "adverse health effects" of PM. In particular, changes in pathways that occur early after particle deposition may or may not be relevant to clinical effects observed hours or even days later.

3. *Choosing appropriate study populations*. Humans are, of course, the most relevant species in which to perform experiments. Human exposure studies can provide dose-response information on some health effects endpoints, help to identify potentially sensitive individuals or populations, and investigate interactions among pollutants. Such studies are limited, however, to comparatively noninvasive health endpoints, small groups of subjects, relatively short exposure durations, and pollutant concentrations that are expected to produce only mild, transient responses. Inhalation studies in animals can include a much broader exposure concentration range and also evaluate a wide variety of physiological, biochemical, and histological endpoints that require invasive techniques not suitable for human studies. Thus, it is possible to gather much more detailed information in animal studies than in human studies, but that information may have low relevance to humans.

Laboratory animals with a particular condition or disease (for example, animals with aspects of human asthma or genetically determined conditions such as hypertension) that are thought to mimic human diseases are available as models in which to study a possibly increased sensitivity to PM. Such models may be useful; however, the relevance to humans of results obtained in animal models is frequently questioned. Different species differ in distinct ways, so it is challenging to extrapolate to humans the results obtained in healthy rodents or dogs, for example. Moreover, animal models of human disease rarely possess all the characteristics of the intended human counterparts, so extrapolating the results to humans is difficult and uncertain.

4. *Using different exposure conditions*. Many types of exposure studies have been conducted. Controlled exposure to single PM components (such as metals or carbon particles of different sizes) has been evaluated in nonhumans. However, most such studies have been conducted at exposure levels much higher than ambient. Although these studies provide an indication of whether a component of the PM mix may have a biologic effect, it may be difficult to extrapolate results to levels of the component found in ambient air. In addition, many of these studies have exposed animals to a single large bolus of particles by intratracheal instillation, bypassing the upper airways. Results obtained by this technique may differ from those obtained by inhalation, the major physiologic route of exposure to particles. Some recent controlled exposure studies have been performed in humans: people with asthma and healthy individuals have been exposed for short periods via inhalation to such PM emissions as diesel exhaust and concentrated ambient particles. Other informative studies have evaluated the effects of administering resuspended particles into the airways of humans. The particles had previously been collected on filters on different occasions at a single ambient monitoring site, making it possible to ascribe differences in airway response to differences in particle composition at the time of collection.

The effects of different sizes of particles have also been compared in experimental and in vitro studies (18,19). In an HEI-funded study, *Günter Oberdörster* and colleagues confirmed their earlier findings that intratracheal instillation of resuspended ultrafine titanium oxide particles induced more of an inflammatory response than did fine particles of the same composition (18). Few studies, however, have compared effects of exposure to fine and ultrafine particles by inhalation, the exposure method that occurs naturally. In their HEI study, *George Leikauf* and colleagues found that inhaled fine nickel sulfate particles induced as great an inflammatory response as inhaled ultrafine nickel sulfate particles (20). The different results found in these two HEI studies may reflect differences in the solubility of particles in the lung (nickel sulfate is water soluble; titanium oxide is not). Other explanations are also possible.

Composition

Metals comprise a large percentage of the urban air PM mass in many countries. Several studies in humans and other species have identified a potential role of metals in the induction of PM-related effects. One recent study (21) combined epidemiologic and experimental approaches. Particles collected from a monitoring site in the Utah Valley at different times were resuspended and administered to the airways of human volunteers. Particles collected during a period in which metal levels (specifically iron, copper, zinc, lead, and nickel) in the particles were high (local steel mill running) induced a greater inflammatory response in the lungs than when metal levels were low (steel mill closed). Short-term exposure of rodents to high concentrations of nickel and vanadium or of residual oil fly ash induced inflammatory, respiratory, and cardiovascular responses, including cardiac arrhythmias (22,23). (Residual fly ash is an emission from power plants that is rich in particles containing metals, especially iron, nickel, and vanadium. It contains metals at levels and proportions much higher than those found in ambient air.)

In HEI studies currently under review, *Ann Aust* and colleagues showed that iron can detach from coal fly ash taken into airway epithelial cells growing in culture and stimulate the production of mediators that induce inflammatory responses (71). *Fletcher Hahn* and colleagues have exposed old rats and rats with preexisting airway inflammation to fine and ultrafine vanadium pentoxide particles and to similar-sized carbon particles and compared the effects of these particles on lung inflammation and disease. This study should provide valuable information about the effects of both particle size and particle composition (72). In their ongoing HEI study, *Kent Pinkerton* and colleagues are investigating the effects of ultrafine iron particles, inhaled alone or in combination with carbon particles, in young and adult rats on a number of endpoints, including the pattern of injury in the lower respiratory tract.

Ambient air also contains many different organic compounds associated with combustion particles. However, much less work has been done to investigate the health effects of these compounds than to investigate the health effects of metals. Some studies have shown that an organic fraction extracted from diesel exhaust particles, an emission reported to enhance the induction of at least some of the characteristics of the allergic response in humans and other species, enhances the synthesis of immunoglobulin E in vitro (24,25). (Immunoglobulin E is a key mediator of the allergic response.) In addition, a similar organic extract of diesel exhaust particles has been reported to have cytotoxic effects in macrophages and epithelial cells in vitro (26).

Some experimental and epidemiologic studies have tried to associate health effects not only with specific components of PM but also with specific sources of particles (10,27–29). The statistical approaches in these studies, which included factor analysis and principal component analysis, are based on assumptions about the groups of elements that characterize an emission source.

How Do Particles Cause Effects?

One of the major advances in PM research in the last few years is the tentative identification of plausible biologic mechanisms to explain the epidemiologic findings of associations between increased exposure to PM and increased mortality. Many of the findings from studies using particles comparable to or derived from those found in ambient air (in particular, concentrated ambient particles [CAPs]) and from studies in populations particularly susceptible to the effects of PM. Many of the new findings are early

events that occur within minutes or hours after exposure to particles. The relevance of changes in these parameters to the subsequent development of short- or long-term adverse health effects is still not clear. Moreover, these new biologic data do not definitively establish the sequence of events that occurs after particles deposit in the airways. Nonetheless, these findings present a credible view of how even low-level exposure to PM may alter the cardiovascular and pulmonary systems and pose a particular threat to people with cardiovascular or respiratory conditions.

These recent findings pertain to: the induction of inflammatory responses in the airways; the induction of systemic inflammatory and other vascular responses; and changes in neural control of heart function. Figure 2 summarizes the overlapping pathways by which deposition of particles in the airways can induce effects both in the airways and throughout the body (systemically) that may lead to adverse effects in the airways and the cardiovascular system.

Inflammatory Responses in the Airways

Recent controlled-exposure studies in humans indicate that different types of particles can induce an inflammatory response in the airways, the site at which particles first deposit (30–32). This is measurable in a number of ways, including an increase in neutrophil number and in levels of cytokines and chemokines associated with the inflammatory

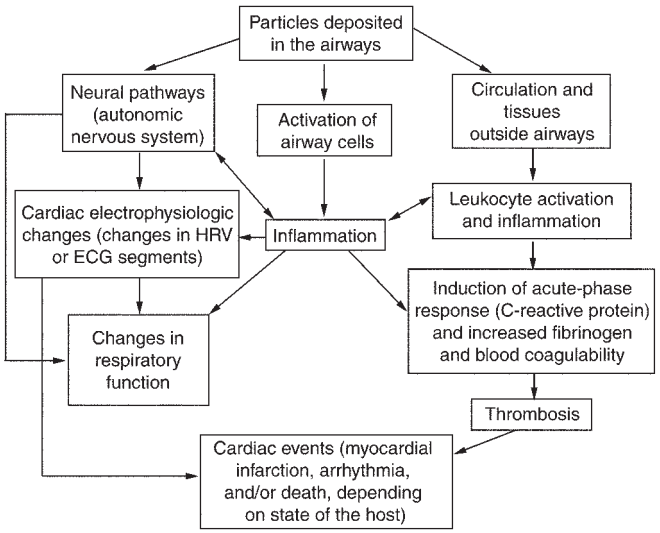

Figure 2. How PM can affect the airways and the cardiovascular system. (HRV = heart rate variability.)

response. In an HEI study currently under review, *Stephen Holgate* and colleagues have extended their original studies in which they found airway inflammation in healthy subjects after exposure to diesel exhaust (31,32). They evaluated in healthy people and in those with mild asthma the effects of exposure to a lower concentration of diesel exhaust than they had used in earlier studies. They found small increases in inflammatory markers (such as lung neutrophil levels) in healthy but not asthmatic subjects (70).

Experiments in animals and in vitro have shown that the metal and organic components of PM can induce inflammatory cytokine and chemokine formation as the end result of *oxidative stress* pathways inside cells. These pathways generate what are known as reactive oxygen species, including free radicals, hydrogen peroxide, and superoxide (26,33). As described in a previous section, the induction of an inflammatory response by PM in the airways may damage not only the epithelial cell layer at the surface of the tissue but also other airway cells such as macrophages. Some recent data support this hypothesis. For example, exposing a macrophage cell line to fine and ultrafine particles decreased phagocytosis (the ability of scavenger cells to engulf and remove material from the extracellular milieu) (19).

Airway nerve cells may also contribute to inflammation in the airways by synthesizing neurotransmitters (75). In this *neurogenic inflammation*, the neurotransmitters may affect many types of white blood cells in the lung, as well as epithelial and smooth muscle cells. Inflammatory cytokines synthesized by white blood cells may also affect the nerve cells.

One possible consequence of damage to the airways is that the individual may become more susceptible to respiratory infections if exposed to viruses or bacteria (discussed in 34). A second possible consequence is that it may decrease respiratory function in a person whose airways are already damaged by conditions such as bronchitis or asthma. As a result, the symptoms of asthma, for example, may be exacerbated.

Systemic Inflammatory and Other Vascular Responses

Recent studies have suggested that exposure to particles results in systemic inflammatory effects within hours

after exposure. It is currently not clear if the systemic response is a consequence of an inflammatory response in the airways, because some studies have detected little or no inflammatory response after exposure to PM. As described earlier, some studies indicate that either particles per se (and ultrafine particles in particular) or components that may detach or dissolve from particles may move rapidly into the bloodstream and reach other tissues (73,74).

One marker of systemic inflammation that has been detected after exposure to PM is an increased number of circulating neutrophils (31, 35, and 29 in humans, rats, and dogs, respectively). Increased bone marrow production of immature neutrophils has also been reported (36,37). Epidemiologic studies have described associations between PM exposure and other vascular factors, and controlled-exposure studies have reported PM-dependent effects on levels of additional vascular factors (31,38–44). These factors include fibrinogen, plasma viscosity, platelet numbers, C-reactive protein, endothelin levels, and blood pressure. Several of these factors (fibrinogen, C-reactive protein, and blood pressure) are independently associated with increased risk of cardiovascular disease, which could affect susceptibility to the acute effects of PM.

The changes in vascular parameters that occur after particle exposure suggest that exposure to PM may lead to higher levels of fibrinogen, in turn increasing plasma viscosity, and the ability of blood to coagulate. This may result in an increased tendency to form clots and thrombi (aggregations of platelets and other blood components causing vascular obstruction). Although the outcome of these phenomena for healthy individuals is not clear, it is probable that inducing clots or thrombi in those with damaged cardiac or vascular systems may have more serious consequences. Individuals with atherosclerosis may be particularly at risk. Atherosclerosis is characterized by a thickening and hardening of the arteries in which *plaque* (deposits of cholesterol and other fats, plus fibrin and inflammatory cells and factors) narrows the arteries and decreases the arterial blood flow. In atherosclerosis, the functions of endothelial cells, the cells lining the blood vessel, are also impaired. This results in additional production of mediators that promote vasoconstriction, the narrowing of blood vessels.

If a thrombus forms on the plaque's surface, or bleeding into the plaque occurs, the entire artery may become blocked. If this occurs in a coronary artery, the supply of oxygen to the heart muscle is reduced. This condition, myocardial ischemia, may lead to heart damage and *arrhythmias*, disturbances in the rhythmic beating of the heart. Arrhythmias, such as ventricular fibrillation, may have serious and potentially fatal consequences because they can lead to a heart attack (myocardial infarction [MI]). In addition to vascular changes that result in arrhythmias, arrhythmias may also develop as a consequence of changes in the neural control of heart function.

Several studies in humans and other species link exposure to PM with changes in cardiac function, including inducing arrhythmias and increasing the incidence of MIs (22,23,44–48). In their HEI study, *John Godleski* and colleagues induced a temporary coronary occlusion (cutting off blood supply to the heart via the coronary artery) in a small number of dogs and then exposed them to CAPs. They found that exposure to CAPs induced a more rapid and larger elevation in the ST segment on an electrocardiogram (ECG) than did exposure to particle-free air (28). This change in the ST segment is one of the characteristic signs of the onset of myocardial ischemia. Godleski's study was small and follow-up research is required to confirm these results. This finding supports the mechanism by which people with atherosclerotic arteries may be more vulnerable to cardiac problems, such as fatal arrhythmias, when exposed to PM. Kodavanti and colleagues have reported that spontaneously hypertensive rats exposed to residual fly ash also show enhanced changes in the ST segment (49).

In their ongoing HEI study, *Annette Peters* and colleagues are following up the initial findings of a link between short-term exposure to $PM_{2.5}$ and the fairly rapid incidence of MI (46). They are evaluating whether exposure to air pollution, and ultrafine particles in particular, in the hours or days preceding a nonfatal MI could have triggered the infarction. In their ongoing HEI study, *Douglas Dockery* and colleagues are following up the pilot study findings they reported with Annette Peters: In a small group of patients whose arrhythmias were controlled by an implanted cardiac defibrillator (ICD), the triggering of the ICD

was associated with ambient levels of $PM_{2.5}$ and PM_{10} (47). Dockery and colleagues are currently evaluating the association between exposure to PM and ICD discharge in a larger group of cardiac patients in the Boston area.

Recent findings from a study using a rabbit strain in which the animals develop atherosclerosis indicated that short-term PM_{10} exposure induced atherosclerotic lesions to progress to more advanced stages, potentially making the host more vulnerable to an acute coronary event (37). If confirmed in other studies, these findings might suggest a mechanistic link between PM exposure and increased MI incidence.

PM exposure may also affect other vascular parameters. In a pilot HEI study in rats, *Renaud Vincent* and colleagues found that very high levels of three types of particles increased blood levels of endothelins, which are molecules that affect blood pressure by inducing vasoconstriction (42). (The three types of particles tested were particles from ambient Ottawa air gathered and resuspended in air for the laboratory exposure chamber; resuspended Ottawa air particles from which soluble constituents had been removed ["washed particles"]; and resuspended diesel soot.) The resuspended Ottawa particles also caused a small increase in blood pressure, but diesel soot (composed predominantly of carbon) did not. The washed particles had no effect on blood pressure, which indicates that PM components that are removed by washing (such as metals) might be responsible for this effect. People with atherosclerosis, who have narrowed arteries, may be particularly susceptible to any further narrowing of the arteries induced by increases in endothelins.

Neural Control of Heart Function

Data from recent studies indicate that PM exposure can also affect the neural control of heart function. They indicate that PM exposure in older people and those with cardiac disease was associated with decreased heart rate variability (50–52). Heart rate variability reflects a balance between the two opposing arms of the autonomic nervous system's control of the heart, the sympathetic and parasympathetic nerves. (Stimulation of the sympathetic nerves increases heart rate; stimulation of the parasympathetic nerves decreases the heart rate.) Although reduced heart rate variability is associated with worse outcome in individuals with existing cardiac disease, the clinical significance of similar decreases in healthy individuals is unknown.

Mechanisms of Particle Effects: Conclusions

From all these studies, a more complete picture of the cardiac, pulmonary, and vascular effects of PM exposure is emerging. The reported results, however, are not always consistent from study to study. For example, fibrinogen levels were increased in a human controlled exposure to CAPs (30), and were positively associated with PM exposure in Pekkanen and colleagues' epidemiologic study (38), but had a negative association with PM_{10} exposure in Seaton and colleagues' epidemiologic study (40). In addition, the relation between PM and health varies depending upon the investigators' choice of different "lags" in time between PM exposure on a particular day and the day of the observed health endpoint (eg, in the HEI studies by *Wichmann* and *Lippmann* [16,17]). The HEI study by *Harvey Checkoway* and colleagues provides another example of the differences that can be found in results from epidemiologic studies. That study found no link between sudden cardiac death and PM exposure on the day of death or up to five days before death (53). Because the study was conducted in people with no known heart disease, the result suggests that people who do not have heart disease have little or no increased risk of sudden cardiac death as a result of PM exposure. However, this negative finding does not contradict the results from other, previously cited epidemiologic studies showing that people with respiratory or cardiovascular disease are susceptible to the effects of PM exposure.

Differences in results among experimental studies of similar design have also been noted, particularly in studies of PM effects on airway inflammation. For example, the studies discussed earlier by Holgate and colleagues have reported inflammatory responses (31,32), whereas other studies, including results from the same group, detected little or no response (70). Differences in results on different days of a single controlled-exposure study have also been observed; these day-to-day variations have been ascribed to differences in particle composition [28,54]).

Discrepancies among study findings may be expected, however, given the large variety of exposure conditions, sample sizes, populations, range of endpoints, locations, and research methods used to evaluate results from epidemiologic and experimental studies. Questions still remain as to whether the early changes after PM exposure, which may be normal reactions by the body's biologic defense systems to the introduction of a foreign substance, are or are not the first steps in a pathway leading to adverse health effects.

Are Some Individuals or Groups Particularly Susceptible to the Effects of Particles?

Many epidemiologic and toxicologic studies have compared the effects of PM on morbidity or mortality among different groups. Several studies have shown that estimates of short-term PM effects on acute mortality are increased for people with cardiovascular disease (8,11,15,55), preexisting respiratory conditions (2,56), and older people with preexisting respiratory or cardiovascular disease (57–59). For example, *Mark Goldberg* and colleagues' HEI study used the extensive health records compiled by the Quebec government's health insurance program on hospital admissions and doctor visits to evaluate the underlying causes of mortality associated with PM exposure (56). In the Montreal area, three daily measures of PM level (coefficient of haze, and levels of sulfate and $PM_{2.5}$) were associated with mortality from acute lower, but not upper, respiratory disease, any cardiovascular disease, and other nonaccidental causes of death, including diabetes. This innovative study provided additional evidence that people with respiratory or cardiovascular disease are at risk from PM exposure. It also suggested that individuals with other diseases, such as diabetes, may also have an increased risk of mortality when levels of air pollution increase. This is the first study to show that people with diabetes may be particularly sensitive to PM exposure, so these results require corroboration in additional studies.

Other epidemiologic studies have suggested that ambient PM may affect pregnant women and their fetuses and infants. The results include increases in low birth weight and more infants born prematurely (60,61), and an increase in infant and child mortality (62–64).

Some controlled-exposure studies in animals, particularly those with characteristics that mimic certain human cardiac and pulmonary conditions, support the idea that some groups are more sensitive to the effects of PM than others. For example, in their HEI study, *Günter Oberdörster* and colleagues evaluated whether inhaling ultrafine carbon and platinum particles for a short time induced an inflammatory response in the airways of healthy mice and rats; they also evaluated the effects of these particles in mice and rats with pulmonary conditions that modeled inflammatory diseases such as chronic bronchitis and emphysema (18). Using small numbers of animals and high exposure concentrations, the investigators found that a six-hour exposure induced a small inflammatory response in the airways of mice and rats with pulmonary conditions, but healthy young and old mice and rats showed no response.

Several investigators have tried to determine whether susceptibility to different air pollutants is, at least in part, genetically determined (20,65–67). This question has been explored in mice, because large numbers of genetically identical individuals can be obtained easily. In their HEI study, *George Leikauf* and colleagues used several genetic and molecular approaches to preliminarily characterize genes that affect the response of different mice to continuous inhalation exposure to toxic levels of nickel (20). Using a genetic approach known as *quantitative trait locus analysis,* the investigators showed that certain regions on 5 to 6 distinct chromosomes controlled the toxic response; one region correlated more closely than others with the response. Candidate genes in that region included an important lung protein, surfactant protein B, and a cytokine, transforming growth factor α. In addition, the investigators used gene microarray technology to analyze simultaneously the expression of thousands of genes in the lungs of susceptible and resistant mice during exposure to nickel. They found that nickel exposure produced complex effects on the expression of many genes. Similar approaches may be informative about the nature of genes involved in the human response to air pollutants.

Conclusions: Where Do We Go From Here?

As discussed above, results from epidemiologic and experimental studies funded by HEI and other agencies since the mid 1990s have:

1. *Started to identify characteristics of particles that may induce health effects.* Researchers have reported differential effects from particles of different sizes and composition and have described the health effects of PM components, including metals and organic compounds.
2. *Suggested plausible biologic mechanisms that may underlie the reported associations between short-term increases in PM levels and increases in morbidity and mortality.* Researchers have identified possible initial steps in pathways that may be part of such mechanisms as the induction of systemic and airway inflammatory responses, changes in cardiac and vascular parameters, and changes in neural pathways.
3. *Identified certain groups in the population who appear to be at increased risk from exposure to PM.* These include individuals with respiratory or cardiovascular disease, older people, and possibly other groups.

Although progress has been made in understanding the connection between exposure to PM and adverse health effects, many critical aspects are not yet understood. The first concerns mechanisms: Over the last few years, we have moved from a situation in which we lacked a credible pathophysiologic basis that could explain how increases in PM levels might increase morbidity and mortality to a situation in which PM has been reported to evoke responses in multiple plausible pathways to health effects. Further research is needed to determine the relevance of these pathways, and of the endpoints measured, to the induction of health effects at exposure levels similar to ambient levels. Given the complexity of the body's defenses, it is likely that no single mechanism can account for all PM effects; rather, multiple biologic pathways involving multiple tissues may be involved. In addition, because the components of PM are so diverse, it is also possible that different components of PM may preferentially activate distinct pathways.

A second major concern is untangling the complexity and variability of the PM mixture. Progress has been made in identifying the potentially toxic effects of individual components of the PM mixture, but critical questions remain about the size and chemical composition of the components that exert toxic effects. Information is needed in these areas in order to address the important issue of how to control emissions of PM from different sources.

Figure 3 illustrates the complexity of studying PM toxicity by showing a sample of the types of PM components in the air, the range of study subjects of interest, and health endpoints of potential relevance. It also indicates the types of particles, the range of subjects, and the endpoints evaluated in HEI's epidemiologic and controlled-exposure studies. Other organizations have supported studies that would fill in additional spaces in the matrix, but the effects of many components of the PM mixture have not been systematically or exhaustively studied.

To date, studies supported by HEI and others have strengthened evidence about the effects of particles on health. On the basis of this evidence, government agencies in North America and around the world are taking actions to reduce particle emissions from all of the major stationary and mobile sources. However, after these efforts to reduce particle levels have made initial progress, it is likely that even further efforts to reduce emissions will be proposed. To best inform regulatory standards focused on the most toxic particles, the agencies must have the most accurate and complete information possible about whether certain PM components are more closely linked to adverse health effects than other components, and if so, which particles (and sources of those particles) are of the most concern. Over the long term, targeted standards may affect fewer types of emission sources and methods to control specific components of emissions from affected sources could be developed to meet the standards.

Many studies are under way today to develop needed information on the roles that size and chemical composition of particles have in their toxicity. To make significant headway in evaluating which characteristics and sources of particles are of greatest concern, however, results from previous experimental and epidemiologic studies must be followed up more systematically. The NMMAPS project discussed earlier demonstrated that PM has different effects on mortality in different regions of the United States. These differences are likely due, at least partially, to differences in the nature of PM in the different regions. One

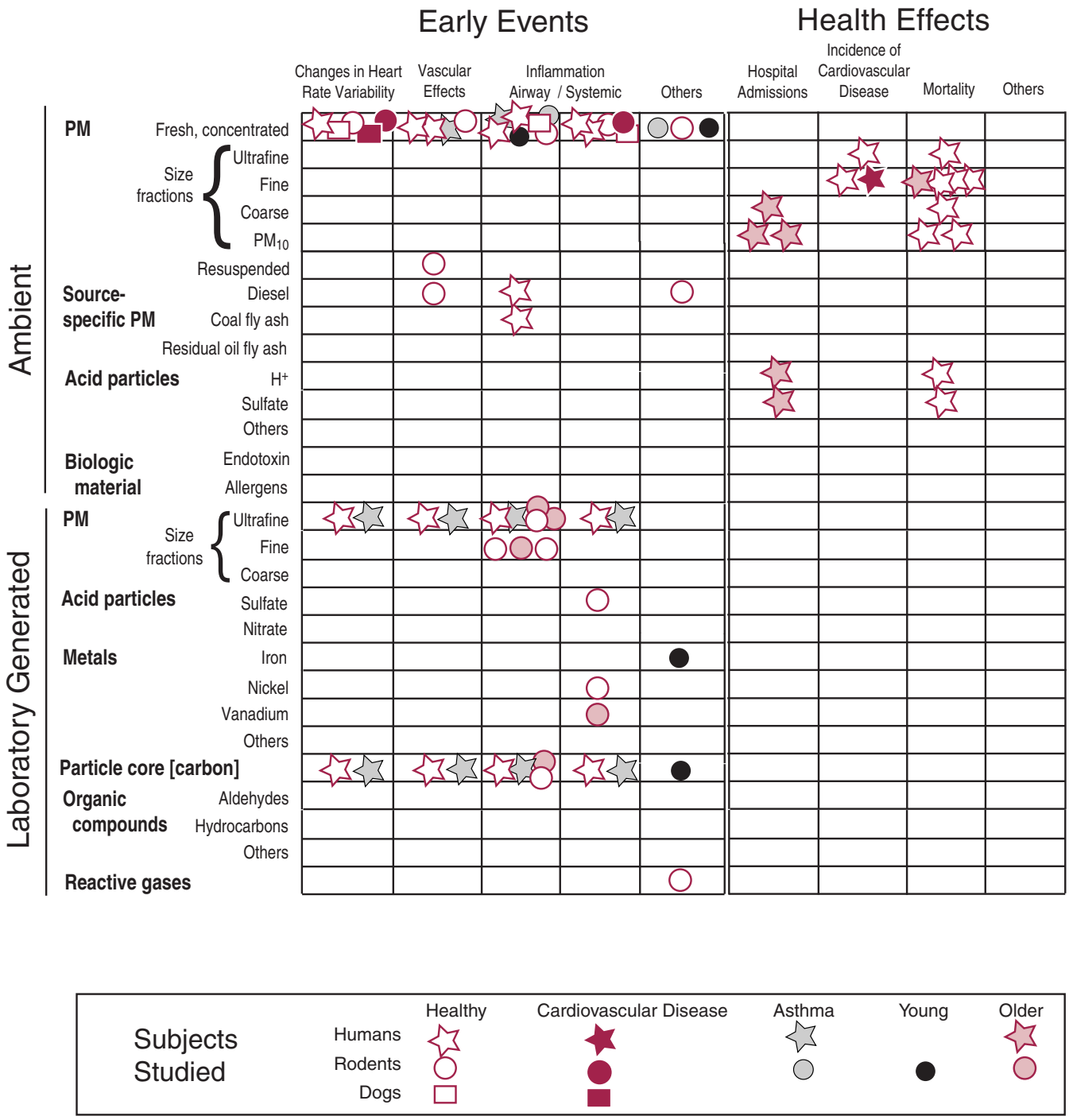

Figure 3. The complexity of studying PM effects shown using studies from HEI's PM research program. The figure illustrates some types of particles, a range of study subjects, and several health endpoints of interest. Each symbol indicates one type of subject that was included in an HEI-funded epidemiologic or experimental study.

A single study may be represented with more than one symbol; for example, the epidemiologic study by Lippmann and colleagues (17) investigated the potential associations among levels of different-sized particles, acidity, and sulfate with daily mortality in the general population and with hospital admissions among older people. For this study and other epidemiologic studies, the general population is represented by the symbol for healthy subjects; the general population, however, also contains susceptible groups.

approach to developing better information about health effects of different types of particles would be to conduct a coordinated set of epidemiologic studies in regions with differences in PM sources and, therefore, in the nature of the particles. Such studies would require detailed characterization of the ambient PM of each region. Some information could be provided by the EPA's Supersite PM monitors and speciation sites, and by readily available high-quality morbidity and mortality databases. A complementary set of experimental studies in animals and humans, with exposure to ambient air in the form of CAPs of different size ranges, could be conducted in the same places and include a much broader array of health endpoints than epidemiologic studies. In addition to CAPs exposure, the experimental studies could include model particles of specific sizes and compositions designed to test hypotheses about specific components of PM in the ambient air. The matrix of subjects studied and the range of endpoints measured in these studies would have to be a carefully selected subset of the larger matrix represented in Figure 3.

Developing a comprehensive coordinated research plan at different sites that builds upon previous work will require a major planning effort; carrying it out will require a substantial investment over a number of years. Nevertheless, providing this information would enable future PM air quality regulations to target the sources of the particles that are most likely to be contributing to adverse health effects. This would ensure that future investments in pollution control will have the largest benefits for public health.

References

1. Dockery DW, Pope CA III. 1994. Acute respiratory effects of particulate air pollution. Annu Rev Public Health 15:107–132.

2. Schwartz J. 1994. What are people dying of on high air pollution days? Environ Res 64:26–35.

3. Katsouyanni K, Karakatsani A, Messari I, Touloumi G, Hatzakis A, Kalandidi A, Trichopoulos D. 1990. Air pollution and cause-specific mortality in Athens. J Epidemiol Commun Health 44:321–324.

4. Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG Jr, Speizer FE. 1993. An association between air pollution and mortality in six US cities. N Engl J Med 329:1753–1759.

5. Pope CA III, Thun MJ, Namboodiri MM, Dockery DW, Evans JS, Speizer FE, Heath CW Jr. 1995. Particulate air pollution as a predictor of mortality in a prospective study of US adults. Am J Respir Crit Care Med 151:669–674.

6. Health Effects Institute. 2001. Airborne Particles and Health: HEI Epidemiologic Evidence. *HEI Perspectives*. Health Effects Institute, Cambridge MA.

7. Schultz H, Brand P, Heyder J. 2000. Particle deposition in the respiratory tract. In: Particle–Lung Interactions, Vol 143, Lung Biology in Health and Disease. Marcel Dekker Inc, New York NY.

8. Schwartz J, Dockery DW, Neas LM. 1996. Is daily mortality associated specifically with fine particles? J Air Waste Manag Assoc 46:927–939.

9. Fairley D. 1999. Daily mortality and air pollution in Santa Clara County, California: 1989-1996. Environ Health Perspect 107:637–641.

10. Burnett RT, Brook J, Dann T, Delocla C, Philips O, Cakmak S, Vincent R, Goldberg MS, Krewski D. 2000. Association between particulate- and gas-phase components of urban air pollution and daily mortality in eight Canadian cities. Inhalation Toxicol 12(Suppl 4):15–39.

11. Ostro BD, Broadwin R, Lipsett MJ. 2000. Coarse and fine particles and daily mortality in the Coachella Valley, California: A follow-up study. J Expo Anal Environ Epidemiol 10:412–419.

12. Castillejos M, Borja-Aburto VH, Dockery DW, Gold DR, Loomis D. 2000. Airborne coarse particles and mortality. Inhalation Toxicol 12(Suppl 1):67–72.

13. Hoek G, Brunekreef B, Verhoeff A, van Wijnen J, Fischer P. 2000. Daily mortality and air pollution in The Netherlands. J Air Waste Manag Assoc 50:1380–1389.

14. Gwynn RC, Burnett RT, Thurston GD. 2000. A time-series analysis of acidic particulate matter and daily mortality and morbidity in the Buffalo, New York, region. Environ Health Perspect 108:125–133.

15. Samet JM, Zeger SL, Dominici F, Curriero F, Coursac I, Dockery DW, Schwartz J, Zanobetti A. 2000. The National Morbidity, Mortality, and Air Pollution Study, Part II: Morbidity and Mortality from Air Pollution in the United States. Research Report 94. Health Effects Institute, Cambridge MA.

16. Wichmann H-E, Spix C, Tuch T, Wölke G, Peters A, Heinrich J, Kreyling WG, Heyder J. 2000. Daily Mortality and Fine and Ultrafine Particles in Erfurt, Germany. Part I: Role of Particle Number and Particle Mass. Research Report 98. Health Effects Institute, Cambridge MA.

17. Lippmann M, Ito K, Nádas A, Burnett RT. 2000. Association of Particulate Matter Components with Daily Mortality and Morbidity in Urban Populations. Research Report 95. Health Effects Institute, Cambridge MA.

18. Oberdörster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder ACP. 2000. Acute Pulmonary Effects of Ultrafine Particles in Rats and Mice. Research Report 96. Health Effects Institute, Cambridge MA.

19. Renwick LC, Donaldson K, Clouter A. 2001. Impairment of alveolar macrophage phagocytosis by ultrafine particles. Toxicol Appl Pharmacol 172:119–127.

20. Leikauf GD, McDowell SA, Wesselkamper SC, Miller CR, Hardie WD, Gammon K, Biswas PP, Korfhagen TR, Bachurski CJ, Wiest JS, Willeke K, Bingham E, Leikauf JE, Aronow BJ, Prows DR. 2001. Pathogenomic Mechanisms for Particulate Matter Induction of Acute Lung Injury and Inflammation in Mice. Research Report 105. Health Effects Institute, Boston MA.

21. Ghio AJ, Devlin RB. 2001. Inflammatory lung injury after bronchial instillation of air pollution particles. Am J Respir Crit Care Med 164:704–708.

22. Watkinson WP, Campen MJ, Costa DL. 1998. Cardiac arrhythmia induction after exposure to residual oil fly ash particles in a

rodent model of pulmonary hypertension. Toxicol Sci 41:209–216.

23. Campen MJ, Nolan JP, Schladweiler MCJ, Kodavanti UP, Evansky PA, Costa DL, Watkinson WP. 2001. Cardiovascular and thermoregulatory effects of inhaled PM-associated transition metals: A potential interaction between nickel and vanadium sulfate. Toxicol Sci 64:243–252.

24. Takenaka H, Zhang K, Diaz-Sanchez D, Tsien A, Saxon A. 1995. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: Direct effects on B-cell IgE production. J Allergy Clin Immunol 95:103–115.

25. Tsien A, Diaz-Sanchez D, Ma J, Saxon A. 1997. The organic component of diesel exhaust particles and phenathrene, a major polyaromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells in vitro. Toxicol Appl Pharmacol 142:256–263.

26. Nel AE, Diaz-Sanchez D, Li N. 2001. The role of particulate pollutants in pulmonary inflammation and asthma: Evidence for the involvement of organic chemicals and oxidative stress. Curr Opin Pulm Med 7:20–26.

27. Laden F, Neas LM, Dockery DW, Schwartz J. 2000. Association of fine particulate matter from different sources with daily mortality in six US cities. Environ Health Perspect 108:941–947.

28. Godleski JJ, Verrier RL, Koutrakis P, Catalano P. 2000. Mechanisms of Morbidity and Mortality from Exposure to Ambient Air Particles. Research Report 91. Health Effects Institute, Cambridge MA.

29. Clarke RW, Coull B, Reinisch U, Catalano P, Killingsworth CR, Koutrakis P, Kavouras I, Krishna Murthy GG, Lawrence J, Lovett E, Wolfson JM, Verrier RL, Godleski JJ. 2000. Inhaled concentrated ambient particles are associated with hematologic and bronchoalveolar lavage changes in canines. Environ Health Perspect 108:1179–1187.

30. Ghio AJ, Kim C, Devlin RB. 2000. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. Am J Respir Crit Care Med 162:981–988.

31. Salvi S, Blomberg A, Rudell B, Kelly F, Sandström T, Holgate ST, Frew A. 1999. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med 159:702–709.

32. Salvi S, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, Wilson S, Sandström T, Holgate ST, Frew AJ. 2000. Acute exposure to diesel exhaust increases IL-8 and GRO-a production in healthy human airways. Am J Respir Crit Care Med 161:550–557.

33. Donaldson K, Brown DM, Mitchell C, Dineva M, Beswick PH, Gilmour P, MacNee W. 1997. Free radical activity of PM_{10}: Iron-mediated generation of hydroxyl radicals. Environ Health Perspect 105(Suppl 5):1285-1289.

34. Gilmour MI, Daniels M, McCrillis RC, Winsett D, Selgrade MJK. 2001. Air pollutant-enhanced respiratory disease in experimental animals. Environ Health Perspect 109(Suppl 41):619–622.

35. Gordon T, Nadziejko C, Schlesinger R, Chen LC. 1998. Pulmonary and cardiovascular effects of acute exposure to concentrated ambient particles in rats. Toxicol Lett 96–97:285–288.

36. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. 1997. Phagocytosis of small carbon particles (PM_{10}) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. Am J Respir Crit Care Med 155:1441-1447.

37. Suwa T, Hogg JC, Quinlan KB, Ohgami A, Vincent R, van Eeden SF. 2002. Particulate air pollution induces progression of coronary atherosclerosis. J Am Coll Cardiol 39:935–942.

38. Pekkanen J, Brunner EJ, Anderson HR, Tiittanen P, Atkinson RW. 2000. Daily concentrations of air pollution and plasma fibrinogen in London. Occup Environ Med 57:818–822.

39. Peters A, Döring A, Wichmann H-E, Koenig W. 1997. Increased plasma viscosity during an air pollution episode: A link to mortality? Lancet 349:1582–1587.

40. Seaton A, Soutar A, Crawford V, Elton R, McNerlan S, Cherrie J, Watt M, Agius R, Stout R. 1999. Particulate air pollution and the blood. Thorax 54:1027–1032.

41. Schwartz J. 2001. Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109(Suppl 3):405–409.

42. Vincent R, Kumarathasan P, Goegan P, Bjarnason SG, Guénette J, Bérubé D, Adamson IY, Desjardins S, Burnett RT, Miller FJ, Battistini B. 2001. Inhalation Toxicology of Urban Ambient Particulate Matter: Acute Cardiovascular Effects in Rats. Research Report 104. Health Effects Institute, Boston MA.

43. Peters A, Frohlich M, Döring A, Immervoll T, Wichmann H-E, Hutchinson WL, Pepys MB, Koenig W. 2001. Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198–1204.

44. Ibald-Mulli A, Stieber J, Wichmann H-E, Koenig W, Peters A. 2001. Effects of air pollution on blood pressure: A population-based approach. Am J Public Health 91:571–577.

45. Poloniecki JD, Atkinson RW, de Leon AP, Anderson HR. 1997. Daily time series for cardiovascular hospital admissions and previous day's air pollution in London, UK. Occup Environ Med 54:535–540.

46. Peters A, Dockery DW, Muller JE, Mittleman MA. 2001. Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810–2815.

47. Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh JA, Allen G, Monahan K, Dockery DW. 2000. Air pollution and incidence of cardiac arrhythmia. Epidemiology 11:11–17.

48. Campen MJ, Costa DL, Watkinson WP. 2000. Cardiac and thermoregulatory toxicity of ROFA in cardiopulmonary compromised rats. Inhalation Toxicol 12(Suppl 2):7–22.

49. Kodavanti UP, Schladweiler MC, Ledbetter AD, Watkinson WP, Campen MJ, Winsett DW, Richards JR, Crissman KM, Hatch GE, Costa DL. 2000. The spontaneously hypertensive rat as a model of human cardiovascular disease: Evidence of exacerbated cardiopulmonary injury and oxidative stress from inhaled emission particulate matter. Toxicol Appl Pharmacol 164:250–263.

50. Liao D, Creason J, Shy C, Williams R, Watts R, Zweidinger R. 1999. Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. Environ Health Perspect 107:521–525.

51. Pope CA III, Verrier RL, Lovett EG, Larson AC, Raizenne ME, Kanner RE, Schwartz J, Villegas GM, Gold DR, Dockery DW. 1999. Heart rate variability associated with particulate air pollution. Am Heart J 138:890–899.

52. Gold DR, Litonjua A, Schwartz J, Lovett E, Larson A, Nearing B, Allen G, Verrier M, Cherry R, Verrier R. 2000. Ambient pollution and heart rate variability. Circulation 101:1267–1273.

53. Checkoway H, Levy D, Sheppard L, Kaufman J, Koenig J, Siscovick D. 2000. A Case-Crossover Analysis of Fine Particulate

Matter Air Pollution and Out-of-Hospital Sudden Cardiac Arrest. Research Report 99. Health Effects Institute, Cambridge MA.

54. Kobzik L, Goldsmith CAW, Ning YY, Qin G, Morgan B, Imrich A, Lawrence J, Krishna Murthy GG, Catalano PJ. 2001. Effects of Combined Ozone and Air Pollution Particle Exposure in Mice. Research Report 106. Health Effects Institute, Boston MA.

55. Schwartz J. 1993. Air pollution and daily mortality in Birmingham, Alabama. Am J Epidemiol 137:1136–1147.

56. Goldberg MS, Bailar JC III, Burnett RT, Brook JR, Tamblyn R, Bonvalot Y, Ernst P, Flegel KM, Singh RK, Valois M-F. 2000. Identifying Subgroups of the General Population That May Be Susceptible to Short-Term Increases in Particulate Air Pollution: A Time-Series Study in Montreal, Quebec. Research Report 97. Health Effects Institute, Cambridge MA.

57. Kelsall JE, Samet JM, Zeger SL, Xu J. 1997. Air pollution and mortality in Philadelphia, 1974-1988. Am J Epidemiol 146:750–762.

58. Ostro B, Sanchez JM, Aranda C, Eskeland GS. 1996. Air pollution and mortality: Results from a study of Santiago, Chile. J Expo Anal Environ Epidemiol 6:97–114.

59. Simpson RW, Williams G, Petroeschevsky A, Morgan G, Rutherford S. 1997. Associations between outdoor air pollution and daily mortality in Brisbane, Australia. Arch Environ Health 52:442–454.

60. Woodruff TJ, Grillo J, Schoendorf KC. 1997. The relationship between selected causes of postneonatal infant mortality and particulate air pollution in the United States. Environ Health Perspect 105:608–612.

61. Dejmek J, Selevan SG, Benes I, Solansky I, Šrám RJ. 1999. Fetal growth and maternal exposure to particulate matter during pregnancy. Environ Health Perspect 107:475–480.

62. Loomis D, Castillejos M, Gold DR, McDonnell W, Borja-Aburto VH. 1999. Air pollution and infant mortality in Mexico City. Epidemiology 10:118–123.

63. Ostro B, Chestnut L, Vichit-Vadakan N, Laixuthai A. 1999. The impact of particulate matter on daily mortality in Bangkok, Thailand. J Air Waste Manag Assoc 49:PM100–PM107.

64. Ritz B, Yu F, Chapa G, Fruin S. 2000. Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. Epidemiology 11:502–511.

65. Kleeberger SR, Levitt RC, Zhang LY, Longphre M, Harkema J, Jedlicka A, Eleff SM, DiSilvestre D, Holroyd KJ. 1997. Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17:475–478.

66. Kleeberger SR, Reddy S, Zhang LY, Jedlicka AE. 2000. Genetic susceptibility to ozone-induced lung hyperpermeability: Role of toll-like receptor 4. Am J Respir Cell Mol Biol 22:620–627.

67. Ohtsuka Y, Brunson KJ, Jedlicka AE, Mitzner W, Clarke RW, Zhang LY, Eleff SM, Kleeberger SR. 2000. Genetic linkage analysis of susceptibility to particle exposure in mice. Am J Respir Cell Mol Biol 22:574–581.

68. Gordon T, Nadziejko C, Chen LC, Schlesinger R. 2000. Effects of Concentrated Ambient Particles in Rats and Hamsters: An Exploratory Study. Research Report 93. Health Effects Institute, Cambridge MA.

69. Environmental Protection Agency (US). 1996. Air Quality Criteria for Particulate Matter, Vol III. EPA/600/P-95/001CF. National Center for Environmental Assessment, Research Triangle Park NC.

70. Holgate ST, Sandström T, Frew AJ, Stenfors N, Nördenhall C, Salvi S, Blomberg A, Helleday R, Söderberg M. In review. The Health Effects of Acute Exposure to Diesel Exhaust and Concentrated Ambient Particles. Part I: Exposure of Normal and Asthmatic Subjects to Fresh Diesel Exhaust. Health Effects Institute, Boston MA.

71. Aust A, Smith KR, Veranth JM, Hu A, Lighty JS, Ball JC, Stracci AM, Young WC. 2002. Particle Characteristics Responsible for Effects on Human Lung Epithelial Cells. Health Effects Institute, Boston MA. In press.

72. Hahn F, Barr EB, Ménache MG, Seagrave JC, Nikula KJ. In review. Mechanisms of Particle Size- and Composition-Related Adverse Health Effects in an Aged, Sensitive Population of Rats. Health Effects Institute, Boston MA.

73. Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B. 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105:411–414.

74. Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PH, Verbruggen A, Nemery B. 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am J Respir Crit Care Med 164:1665–1668.

75. Barnes PJ. 2001. Neurogenic inflammation in the airways. Respir Physiol 125:145–154.

76. Laskin DL, Morio L, Hooper K, Li T-H, Buckley B, Turpin B. 2002. Role of Peroxides and Macrophages in Fine Particulate Matter Toxicity. Health Effects Institute, Boston MA. In press.

77. Nadziejko C, Chen LC, Cohen B, Karpatkin M, Nadas A. 2002. Effects of Concentrated Ambient Particulate Matter on Blood Coagulation Parameters in Rats. Health Effects Institute, Boston MA. In press.

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Phone +1-617-886-9330 Fax +1-617-886-9335 pubs@healtheffects.org www.healtheffects.org